CUNI-CZ

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Platform: Windows

Prerequisites: MATLAB Compiler Runtime 2009a (x86)

CUNI-CZ: SUMMARY

Automated segmentation of objects like cells or cell nuclei and their tracking in time is an important procedure for biological research. For segmentation, we propose a simple method based on the *k*-means threshold selection algorithm combined with a sliding neighborhood approach. For tracking of segmented objects, we applied a simple method based on frame-by-frame association of nearest neighbors. The algorithm was originally designed for data from our Leica SP5 confocal and Andor spinning disk microscopes and is based on the experience the authors developed in previous projects.

CUNI-CZ: PREPROCESSING

Each raw camera image is slightly blurred by a Gaussian kernel with a user-defined standard deviation σ. We perform this step in order to reduce noise and to smooth out the boundaries of segmented objects. If one z-stack consists of more images, then blurring is performed for each image in the z-stack separately and a maximal intensity projection is used as the final image.

CUNI-CZ: SEGMENTATION

The segmentation algorithm starts with threshold estimation. We applied an iterative threshold selection method based on the *k*-means algorithm [1] to the blurred camera image. In order to cover large intensity variations of cells and background, the threshold value is appropriately adopted in different parts of the image. We did this using a sliding neighborhood approach with a window size (*box*) given by the user and with a step of a half of the window size. The threshold value is estimated in each of the sliding windows. In this way, we obtain a discrete map of thresholds for different parts of the image. A smooth threshold map is obtained by bi-cubic interpolation of the discrete map.

A binary image with masks of the cells is obtained by thresholding the blurred camera image using the generated smooth threshold map. After thresholding, we apply a fill hole transform [2] on the binary image to fill regions with holes. Next, to split masks of cells belonging to one connected component (i.e., to separate two touching cells), we perform a watershed transform [3] computed on images obtained by

one of the following two strategies. Either morphological opening by reconstruction [4] of the blurred camera image; or morphological opening by reconstruction of a distance transform [5] of the thresholded binary image. The size of the structuring element for the morphological opening operation is a user-defined parameter. Finally, cells are detected as connected components in the final binary image. Only connected components larger than a given minimum area (*minArea*) are taken into account.

CUNI-CZ: TRACKING

The tracking algorithm is based on the nearest neighbor approach. As a reference point for each segmented object, we used its center of gravity. We measured the pair-wise distances between the positions of the objects in the current frame and the positions of the objects in the previous frames. All distances are transformed to probabilities, with higher values corresponding to better position matches. With each object, we also associated a weight w such that $w = 1$ for an object which is still in the field of view, $w = 0$ for an object that divided into more objects so we do not need to track it further, and $w \in (0,1)$ if the object disappears from the field of view. Combining probabilities with weights (*distNorm*) and the user-defined threshold (*distThres*), we can distinguish the following situations:

- Many-to-one case (multiple cells overlap): Object in the current frame is marked with the label of the closest object from the previous frame. For other objects in the neighborhood we follow the one-to-none case.
- One-to-one case (object migrates to a new position): We keep the track of the object and update its position.
- One-to-none case (object disappears from the field of view): The position cannot be updated, but the weight of the object is decreased by 5%.
- None-to-one case (object enters the field of view): A new object is created.
- One-to-many case (object divides into two or more objects): Two or more new objects are created, and for the original object we set the weight to zero.

CUNI-CZ: POST-PROCESSING

No post-processing step is performed.

REFERENCES

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